PATENTS • DESIGNS • COPYRIGHT • TRADE MARKS
The Patent Office

#4

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 5 March 2001

Patents Form 1/77

THE PATENT OFFICE
02 JUL 1998
RECEIVED BY FAX

The Patent Office

1/77

03.JUL98 E372944-4 D00056
P01/7700 25.00 - 9814380.3

Patents Act 1977
(Rule 16)

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

MPW/P21121GB

2. Patent application number
(The Patent Office will fill in this part)

9814380.3

-2 JUL 1998

3. Full name, address and postcode of the or of each patent applicant *(underline all surnames)*

EISAI LONDON RESEARCH LABORATORIES LIMITED
BERNARD KATZ BUILDING
UNIVERSITY COLLEGE LONDON
GOWER STREET
LONDON WC1E 6BT
UK

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

6347256001

4. Title of the invention

INVENTION

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

KILBURN & STRODE
20 RED LION STREET
LONDON
WC1R 4PJ

Patents ADP number *(if you know it)*

125001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application number	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

YES

a) any applicant named in part 3 is not an inventor, or

b) there is an inventor who is not named as an applicant, or

c) any named applicant is a corporate body. See note (d))

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	:	
Description	:	8½
Claim(s)	:	1½
Abstract	:	1
Drawing(s)	:	(see pages 4 and 5)

10. If you are also filing any of the following, state how many against each item.

Priority documents	:
Translations of priority documents	:
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	:
Request for preliminary examination and search *(Patents Form 9/77)*	:
Request for substantive examination *(Patents form 10/77)*	:
Any other documents *(please specify)*	:

11. I/We request the grant of a patent on the basis of this application.

Signature: Kilburn & Strode

Date: 2 July 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

Martin P White
Tel: 0171-242 8291

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered `Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

INVENTION

Introduction

The majority of excitatory synaptic responses in mammalian CNS are elicited by amino acids such as L-glutamate or L-aspartate and are mediated by four different receptor subtypes. Three of these receptors are coupled to ionophores and are known as the N-methyl-D-aspartate (NMDA), the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate), and the kainate receptors. The fourth receptor subtype is linked to phosphoinositol metabolism and is known as the metabotropic glutamate receptor.

The NMDA receptor is coupled to high conductance channels permeable to Na^+, K^+, and Ca^{2+} (McBain and Mayer, 1994). It is modulated by glycine (coagonist) and polyamines (positive modulator) and is blocked in a use- and voltage dependent manner by Mg^{2+}. The functional NMDA receptor is thought to be formed as a pentameric subunit assembly consisting of subunit selection from NR1 (eight isoforms) and NR2 (four isoforms) families (Hollmann and Heinemann, 1994). The type of subunits forming the NMDA channel determine its biophysical properties and physiological function (Schöpfer et al., 1994). The AMPA receptor is permeable to Na^+ and K^+ (Hollmann and Heinemann, 1994). AMPA receptor-dependent ion channel is formed from four different subunits designated as GluR1 to GluR4 (in two alternative splice variants - flip and flop) in a tetrameric subunit assembly (Hollmann and Heinemann, 1994; Rosenmund et al., 1998). Pharmacological properties of AMPA receptor-dependent ion channels are determined by the selection of subunits. Channel assemblies lacking GluR2 subunits are permeable to Ca^{2+} in addition to Na^+- and K^+-permeability (Hollmann and Heinemann, 1994). In situ hybridization has revealed different expression of glutamate receptor subunits throughout the brain and during development (Monyer, et al., 1994).

In contrast to the well documented role of glutamate in the pathogenesis of neuronal degeneration resulting from hypoxia/ischemia, hypoglycemia, convulsions and head or spinal cord trauma, it has been difficult to establish a link between glutamate-mediated cell death and demyelinating disorders. Here we provide evidence

suggesting a novel concept for the involvement of glutamate in the pathogenesis of demyelinating disorders which establishes a link between neuronal demyelination and glutamate-mediated cell death and suggest that glutamate antagonists may prove beneficial in the treatment of demyelinating diseases that have been resistant to therapy.

Until recently, treatment of human demyelinating conditions relied exclusively on immunosupressive agents such as corticosteroids and cyclophosphamide, providing limited benefit to patients irrespective of the side effects associated with the long-term treatment. The introduction of interferon preparations has provided efficacy in the treatment of the demyelinating disorders, but is still insufficient in the management of these diseases.

Experimental allergic encephalomyelitis (EAE), an iducible autoimmune disease, represents the best characterized animal model of a demyelinating disorder and drugs active in this model proved to be active in humans (Pender 1996).

Here we describe a surprising observation on the reduction in neurological deficits during acute EAE in rats following treatment with a non-immunomodulatory and non-antiinflamatory agent, the AMPA receptor antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo-(F)-quinoxaline (NBQX), as an example of an agent modulating the function of the AMPA receptor complex.

Methods

Animals

Female Lewis rats (205 ± 10 g) obtained from Charles River, Kent UK, were housed in pairs under environmentally controlled conditions (6:00 a.m. - 6:00 p.m. light/dark cycle; 22-24°C; 45-55% humidity) and allowed free access to food and water. Experimental groups consisted of 10 animals.

Induction of Acute-Active EAE in Lewis Rats

Rats were immunised in each hind foot with 50 µl of inoculum containing 50 µg guinea pig myelin basic protein (MBP, prepared by the method of Dunkley and Carnegie (1974); final concentration 2 mg/ml), emulsified in Freund's complete adjuvant (CFA; Sigma, UK) containing Mycobacterium tuberculosis H37Ra (final concentration 5.5 mg/ml; Difco Laboratories, UK).

Assessment of Clinical EAE in Lewis rats

Animals were weighed and monitored daily and clinical disease scored as (0) no clinical signs; (1) flaccid tail and weight loss; (2) hind limb hypotonia with further weight loss; (3) complete hind limb paralysis; (4) paraplegia and (5) death. In addition, intermediate scores were assigned to animals which showed a loss of tonicity in the distal half of the tail (score = 0.5), paralysis of one hind limb (score = 2.5) or complete hind limb paralysis with forelimb weakness (score = 3.5). During the period of compound administration (10-16 days post immunisation; dpi) animals were scored 15h after injection of vehicle or NBQX to avoid any acute effect of treatment on disease score.

NBQX administration regime

NBQX was initially dissolved in NaOH and diluted with water. pH was adjusted with HCl. Rats were injected i.p. twice daily (9 a.m. and 5 p.m.) on days 10 to 16 post immunisation with either vehicle or NBQX in the dose of 30mg/kg.

Results

Effect of NBQX on disease progression during EAE in the Lewis rat

Following immunisation with MBP, neurological deficit developed in 10/10 vehicle treated animals, 8 of which displayed paralysis of one or both hind limbs; the mean disease onset and duration were 11.8 dpi and 4.7 dpi respectively (Figure 1 and Table 1). Twice daily treatment from day 10 to 16 post immunisation with NBQX completely prevented the development of paralysis in 6 out of 10 rats, whilst one animal exhibited

loss of tone in the most proximal part of the tail (score 0.25) for one day only. The remaining 3 rats displayed paresis of score 1, 2.5 and 3, the onset and duration of which were similar to vehicle injected animals. Thus NBQX significantly reduced disease duration ($p<0.001$), and peak and cumulative disease score ($p<0.01$) relative to vehicle treatment. NBQX also conferred protection on weight loss, significantly delaying the onset until 13 dpi ($p<0.01$) and decreasing the percent body weight lost at the cessation of NBQX administration (day 16; Figure 2 and Table 1).

Figure 1: The AMPA receptor antagonist NBQX reduces severity of paralysis during EAE in rats. NBQX (30mg/kg i.p. twice daily; 10-16 dpi) significantly reduces the peak disease score. Data represents the mean ± SEM of disease score (n=10/group).

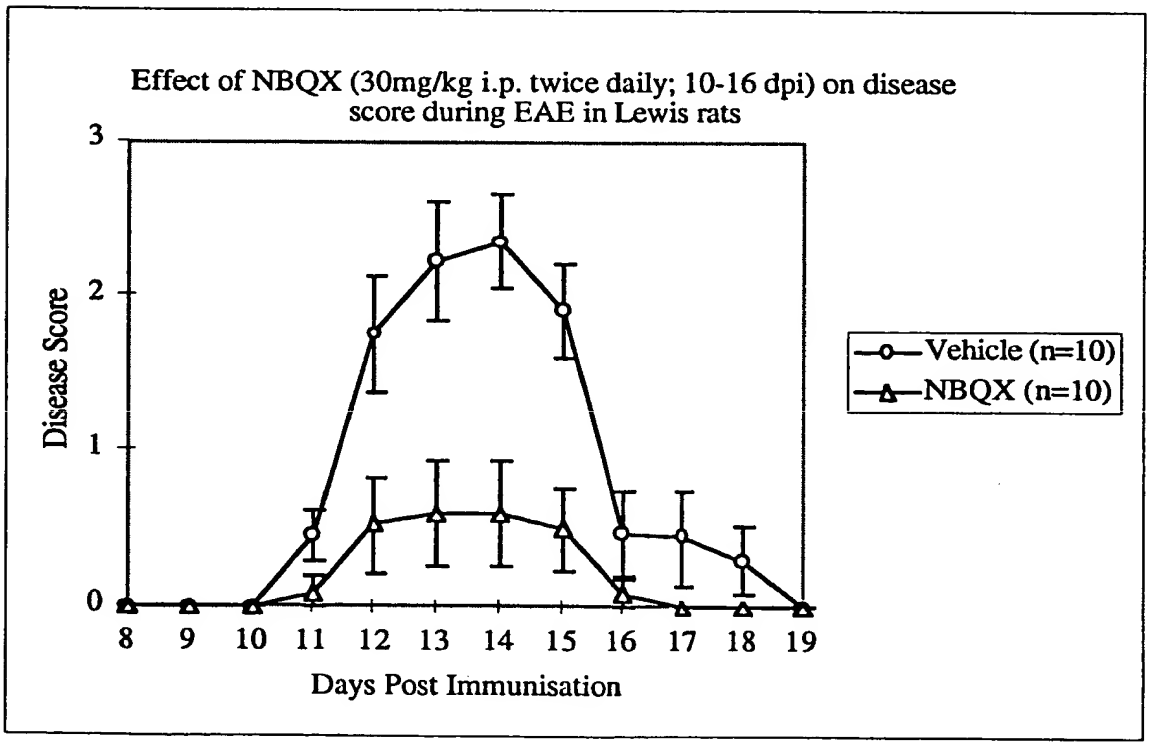

Figure 2: NBQX (30mg/kg i.p. twice daily; 10-16 dpi) reduces weight loss during the course of EAE in rats prior to cessation of treatment (16 dpi). Data represent the mean ± SEM of disease score (n=10/group).

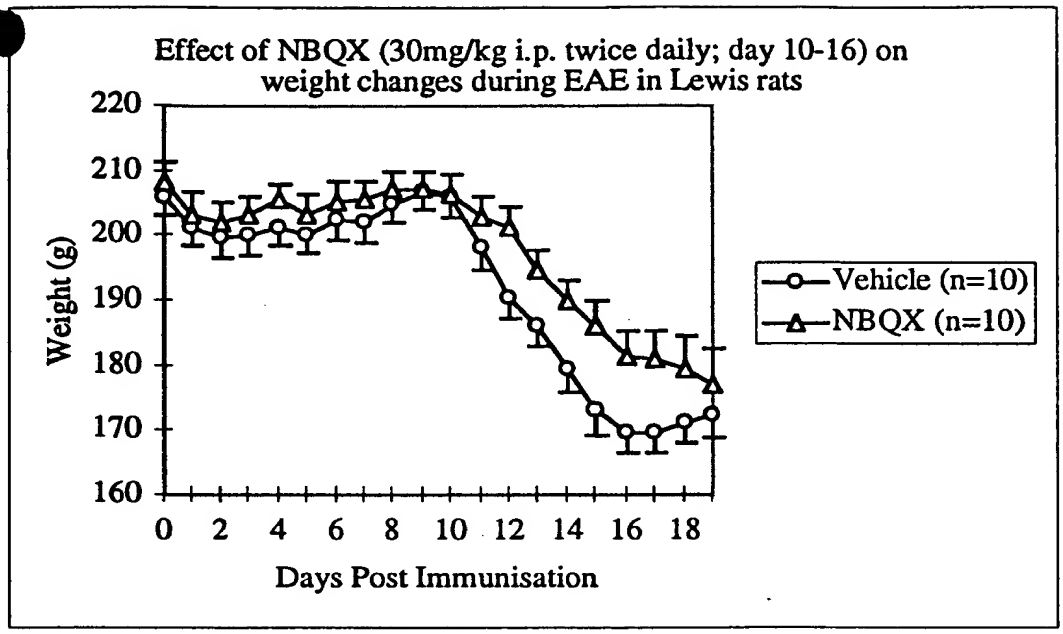

Table 1: Parameters of disease activity during Lewis rat acute EAE.

Treatment	Incidence (%)	[a]Onset (d.p.i.)	Duration (days)	Peak Disease Score	[b]Cumulative Disease Score	[c]Weight Loss (%)
Vehicle	10/10 (100)	11.8 (11-14)	4.7 (4-5)	2.7 (2-3.25)	9.8 (5.5-13)	18 (12-23)
NBQX	4/10 (75)	11.8 (11-12)	1.5 (0-5)††	0.7 (0-3)†	2.4 (0-11.5)†	14 (5-20)*

Values in the table represent the mean and range where n=10; *$p<0.05$, †$p<0.01$ and ††$p<0.001$ vs vehicle, Student t-test or Mann-Whitney U-test for parametric and non-parametric data respectively. a; n=4 for NBQX. b; Cumulative disease score calculated by summation of individual daily disease scores. c; Calculated as the weight on cessation of treatment (16 dpi) expressed as a percent of the maximum weight before disease onset.

Medical uses

1) AMPA antagonists listed in the present invention and exemplified by NBQX may be used in human medicine, although veterinary treatments are not excluded. The treatment may be prophylactic or may be paliative in respect of an existing condition.

2) The AMPA antagonists of the present invention may be used in the manufacture of a medicament for the treatments mentioned above.

3) The medicament will usually be supplied as part of a pharmaceutical composition, which may include a pharmaceutically acceptable carrier.

4) Pharmaceutical compositions within the scope of the present invention may include one or more of the following: preserving agents, solubilising agents, stabilising agents, wetting agents, emulsifiers, sweeteners, colorants, odourants, salts (polypeptides of the present invention may themselves be provided in the form of a pharmaceutically acceptable salt), buffers, coating agents or antioxidants. They may also contain therapeutically active agents in addition to AMPA antaginists of the present invention. If desired, they may be provided in controlled release form.

5) A pharmaceutical composition within the scope of the present invention may be adapted for administration by any appropriate route, for example by the oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) routes. Such a composition may be prepared by any method known in the art of pharmacy, for example by admixing one or more active ingredients with a suitable carrier.

6) Different drug delivery systems can be used to administer pharmaceutical compositions of the present invention, depending upon the desired route of administration.

7) Pharmaceutical compositions adapted for oral administration may be provided as capsules or tablets; as powders or granules; as solutions, syrups or suspensions (in aqueous or non-aqueous liquids); as edible foams or whips; or as emulsions. Tablets or hard gelatine capsules may comprise lactose, maize starch or derivatives thereof, stearic acid or salts thereof. Soft gelatine capsules may comprise vegetable oils, waxes, fats, semi-solid, or liquid polyols etc. Solutions and syrups may comprise water, polyols and sugars. For the preparation of suspensions oils (e.g. vegetable oils) may be used to provide oil-in-water or water-in-oil suspensions.

8) Pharmaceutical compositions adapted for transdermal administration may be provided as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time.

9) Pharmaceutical compositions adapted for topical administration may be provided as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils. For topical administration to the skin, mouth, eye or other external tissues a topical ointment or cream is preferably used. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-water base or a water-in-oil base. Pharmaceutical compositions adapted for topical administration to the eye include eye drops. Here the active ingredient can be dissolved or suspended in a suitable carrier, e.g. in an aqueous solvent. Pharmaceutical compositions adapted for topical administration in the mouth include lozenges, pastilles and mouthwashes.

10) Pharmaceutical compositions adapted for rectal administration may be provided as suppositories or enemas.

11) Pharmaceutical compositions adapted for nasal administration may use solid carriers - eg. powders (preferably having a particle size in the range of 20 to 500 microns). Powders can be administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nose from a container of powder held close to the nose. Compositions adopted for nasal administration may alternatively use liquid carriers – e.g. include nasal sprays or nasal drops. These may comprise aqueous or oil solutions of the active ingredient.

12) Compositions adapted for administration by inhalation may be generated by means of various types of apparatus, eg. pressurised aerosols, nebulizers or insufflators. These apparatuses can be constructed so as to provide predetermined dosages of the active ingredient.

13) Pharmaceutical compositions adapted for vaginal administration may be provided as pessaries, tampons, creams, gels, pastes, foams or spray formulations.

14) Pharmaceutical compositions adapted for parenteral administration include aqueous and non-aqueous sterile injectable solutions or suspensions. These may contain antioxidants, buffers, bacteriostats and solutes which render the compositions substantially isotonic with the blood of an intended recipient. Other components which may be present in such compositions include water, alcohols, polyols, glycerine and vegetable oils, for example. Compositions adapted for parenteral administration may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of a sterile liquid carrier, eg. sterile water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

15) From the above description it will be appreciated that compositions of the present invention can be formulated in many different ways. However preferred compositions of

the present invention are oral, parenteral (intravenous, intramuscular, subcutan) and intrathecal.

16) Dosages of AMPA antagonists of the present invention can vary between wide limits, depending upon the nature of the treatment, the age and condition of the individual to be treated. However, a daily dosage of AMPA antagonists of the present invention of from 0.5 mg to 1000 mg, preferably 50-200 mg may be suitable. The dosage may be repeated as often as appropriate. If side effects develop, the amount and/or frequency of the dosage can be reduced, in accordance with good clinical practice.

Claims

1) Use of competitive and non-competitive AMPA receptor antagonists and AMPA channel blockers and their physiological salts to manufacture as a medicament for the treatment and prevention of demyelinating disorders such as acute disseminated encephalomyelitis, acute demyelinating polyneuropathy (Guillain Barre syndrome), chronic inflammatory demyelinating polyneuropathy, multiple sclerosis, Marchifava-Bignami disease, central pontine myelinolysis, Devic syndrome, Balo disease, HIV- and HTLV-myelopathy, progressive multifocal leucoencephalopathy and in secondary demyelinating disorders such as CNS lupus erythematodes, polyarteriitis nodosa, Sjoegren syndrome, sarcoidosis and isolated cerebral vasulitis.

2) Use of AMPA receptor antagonists and AMPA channel blockers according to claim 1 wherein the AMPA receptor modulating drug is a L-glutamate derivative, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate derivative, quinoline, quinoxaline, quinoxalinedione, quinazolinone, phenylpyridazino-indole-1,4-dione, indeno-pyrazinone, indeno-pyrazine-carboxylic acid, indolo-pyrazinone, imidazo-pyrazinone, amino-phenyl-acetic acid, benzothiadiazine, 4-hydroxypyrrolone, 4-hydroxy-pyrrolo-

pyridazinone, quinolone, amino alkanoic acid, isatin, nitroquinolone, phenyl-azolophthalazine, amino- or desamino- 2,3-benzodiazepine, 2,3-benzodiazepin-4-one, β-carboline-3-carboxylic acid, alkoxy-phenyl-benzodiazepine, acetyl-aminophenyl-dihydro-methyl-dioxolo-benzodiazepine, oxadiazol, isatinoxime, decahydroisoquinoline, sulphamate derivative or its tautomeric form.

3) Use of AMPA receptor antagonists and AMPA channel blockers according to claims 1 and 2 wherein the competitive AMPA antagonist is L-glutamic acid diethylester, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX), 6,7-dinitro-quinoxaline-2,3-dione (DNQX), 6-nitro-7-cyano-quinoxaline-2,3-dione (CNQX), 6-(1-imidazolyl)-7-nitro-quinoxaline-2,3(1H,4H)-dione (YM90K), (3RS,4aRS,6RS,8aRS)-6-(2-(1H-tetrazole-5-yl)ethyl)-decahydroisoquinoline-3-carboxylic acid (LY293558), 9-methyl-amino-6-nitro-hexahydro-benzo(F)quinoxalinedione (PNQX), 8-methyl-5-(4-(N,N-dimethylsulphamoyl)phenyl)-6,7,8,9-tetrahydro-1H-pyrrolo[3,2h]-isoquinoline=2,3-dione-3-O-(3-hydroxybutyric acid-2-yl)oxime (NS1209), 6,7-dichloro-2-(1H)-quinolinone-3-phosphonate (S 17625-2), and [1,2,3,4-tetrahydro-7-morpholinyl-2,3-dioxo-6-(trifluoromethyl)quinoxalin-1-yl]methylphosphonate (ZK 200775), or non-competitve antagonist 1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-5H-2,3-benzodiazepine (GYKI52466), topiramate and 5-{2-[2-(N,N-dimethylamino)ethyl]oxy-phenyl}-3-phenyl-1,2,4-oxadiazol.

4) Use of AMPA receptor antagonists and AMPA channel blockers according to claims 1, 2 and 3 wherein it is combined with immunosuppresive agents (e.g. corticotrophin, glucocorticoids, cyclophosphamide, cyclosporine, azothioprine and mitozantrone), interferon preparations (e.g. Betaseron, Betaferon, Rebif), phosphodiesterase type IV inhibitors, hunmanised monclonal antibodies against leukocyte adhesion molecules (e.g. Antegran), copolymer-1 (e.g. Copaxone), tissue matrix metalloproteinase inhibitors (e.g. BB-3644) or tumour necrosis factor (TNF) inhibitors (e.g. Thalidomide, TNF-receptor immunoglobulin fusion protein) for simultaneous, separate or sequential use.

Abstract

Described is a new use of antagonists acting at the AMPA receptor complex and their physiologically compatible salts for the preparation of drugs for prevention and treatment of neuroimmunological demyelinating disorders such as acute disseminated encephalomyelitis, acute demyelinating polyneuropathy (Guillain Barre syndrome), chronic inflammatory demyelinating polyneuropathy, multiple sclerosis, Marchifava-Bignami disease, central pontine myelinolysis, Devic syndrome, Balo disease, HIV- and HTLV-myelopathy, progressive multifocal leucoencephalopathy and in secondary demyelinating disorders such as CNS lupus erythematodes, polyarteriitis nodosa, Sjoegren syndrome, sarcoidosis and isolated cerebral vasulitis. Also described are pharmaceutical preparations containing these compounds and synergistically active combinations of them with conventional preparations used in the treatment of neuroimmunological disorders.